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AUTHOR(S):

Baba, Naomichi; Kiriata, Mitsunori; Takano, Takenosuke; Ohno, Minoru

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Color Reaction of Pyrethrins and Eugenol with Orthophosphoric, Sulfuric and Hydrochloric Acids*

Naomichi BABA,** Mitsunori KIRIHATA,**
Takenosuke TAKANO*** and Minoru OHNO**

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Color reaction of some alkenyl compounds with orthophosphoric, sulfuric and hydrochloric acids was described and the origin of color was discussed in terms of homoconjugation. Procedure for quantitative analysis of insecticidal constituents in pyrethrum flowers by the color reaction of pyrethrins I and II with orthophosphoric acid reagent was established. As practically applied to the domestic pyrethrum flowers, SHIRAYUKI strain, this method gave the analytical values which compared well with those by the conventional acidimetry.

INTRODUCTION

The importance of pyrethroids as insecticides in particular for domestic use has been growing more and more with the recognition of a global pollution by insecticides. This is due to the advantages of pyrethroids over other hazardous insecticides, in that the pyrethrin-type compounds are by far the less toxic to warm-blooded animals and have even rapid knock-down and/or paralytic effect to various kinds of insects.

In this connection, the necessity has become keen for the method of determination which may enable one to estimate pyrethrins of both natural and synthetic origin as precisely and conveniently as possible on a microgram level.

For this purpose, orthophosphoric acid method was chosen as a most promising candidate for the analysis of natural pyrethrins. This method was proposed firstly by Williams in 1956 and has many merits,¹⁾ however, this bears some ambiguities as to selectivity of the color reaction.

This analytical method is based on the following phenomenon. Insecticidal essence of pyrethrum flowers extracted with organic solvents reacted with an excess of concentrated orthophosphoric acid to give transparent rose red color which is kept considerably stable for several hr. This colored mixture has an absorption maximum at 550 nm and the absorbance at this wavelength is proportional to the amount of pyrethrins with reproducibility. With this color test, one can detect pyrethrins as low a content as 10-microgram order.

Natural "pyrethrins" are a mixture of six insecticidal constituents whose structures closely resemble each other as shown in Table 1. For this reason, it must be revealed that which of these six constituents are responsible for this coloring. In addition, it

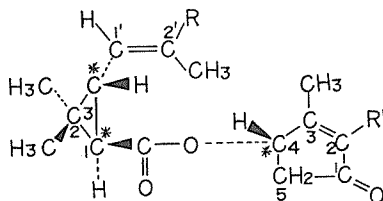
* Taken from the dissertation (N.B.) submitted to Kyoto University (1972, May).

** 馬場 直道, 切畑 光統, 大野 稔: Laboratory of Plant Product Chemistry, Institute for Chemical Research, Kyoto University, Uji, Kyoto.

*** 高野 武之助: The Institute for Japanese Pyrethrum Research. Kyoto University, Uji, Kyoto.

Color Reaction of Pyrethrins

Table 1. Structures of Natural Pyrethrins



(+)-IR:3*R*-*trans* acid-(+)-*S*-*cis*-alcohol ester

| | Constituent | R | R' |
|---|-------------|---------------------|---|
| 1 | Pyrethrin-I | -CH ₃ | $\begin{array}{c} \text{H} \quad \text{H} \\ \quad \\ -\text{CH}_2\text{C}=\text{C}-\text{CH}=\text{CH}_2 \end{array}$ |
| 2 | " -II | -COOCH ₃ | " |
| 3 | Cinerin-I | -CH ₃ | $\begin{array}{c} \text{H} \quad \text{H} \\ \quad \\ -\text{CH}_2\text{C}=\text{C}-\text{CH}_3 \end{array}$ |
| 4 | " -II | -COOCH ₃ | " |
| 5 | Jasmolin-I | -CH ₃ | $\begin{array}{c} \text{H} \quad \text{H} \\ \quad \\ -\text{CH}_2\text{C}=\text{C}-\text{CH}_2\text{CH}_3 \end{array}$ |
| 6 | " -II | -COOCH ₃ | " |

was found out that the mechanism of this coloring could not be explained in terms of simple chromophore, classical carbonium ion formation, coordinate complex formation *etc.* This situation urged us to search for the origin of this phenomenon.

A number of color reactions between mineral acids and organic compounds have been known^{2a-2d)} and widely applied to the detection and quantitative analysis of those compounds, however, the origin of the color development has been obscure. For instance, the Liebermann-Burchard reaction³⁾ has long been used for analysis of steroids, and this color reaction is effected in a mixture of anhydrous acetic acid and concentrated sulfuric acid to give a blueish green color. Despite the fact that this color reaction is specific for the steroidal structure, the mechanism of coloring has not been known at all as yet.

In the present studies, some unsaturated organic compounds were treated with orthophosphoric acid-ethyl acetate, sulfuric acid-anhydrous acetic acid and hydrochloric acid-anhydrous acetic acid. Then, from the viewpoint of the homoconjugation theory, the origin of the color was discussed. In addition, the procedure for pyrethrin assay by means of the coloring with orthophosphoric acid-ethyl acetate (4:1 by volume) reagent was established and applied to the dried pyrethrum flowers.

RESULT AND DISCUSSION

(I) Treatment of Alkenyl Compounds with Mineral Acids

Nine compounds were treated with three types of color reagents. All the materials

and reagents used are transparent over the visible region. Color tests were carried out in the following manner: 5 ml of the color reagent were added to 0.5–5 mg of solvent-free alkenyl compounds and, after mixing, was heated at 100–120°C for 3–5 min. When a color of the mixture developed at all, the UV spectra were taken, using the reagent itself as reference. The results were collected in Table 2, which showed that the behaviors of all the alkenyl compounds except *cis*- and *trans*-isoeugenol towards the three types of reagents were similar. Accordingly, the origin of the color might resemble in every case. Absorption maxima were not the same with each reagent and it may be attributed to differences in some natures of solvent.

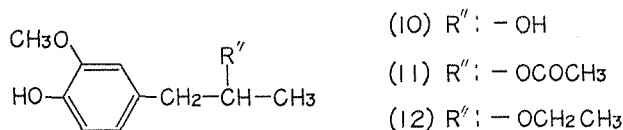
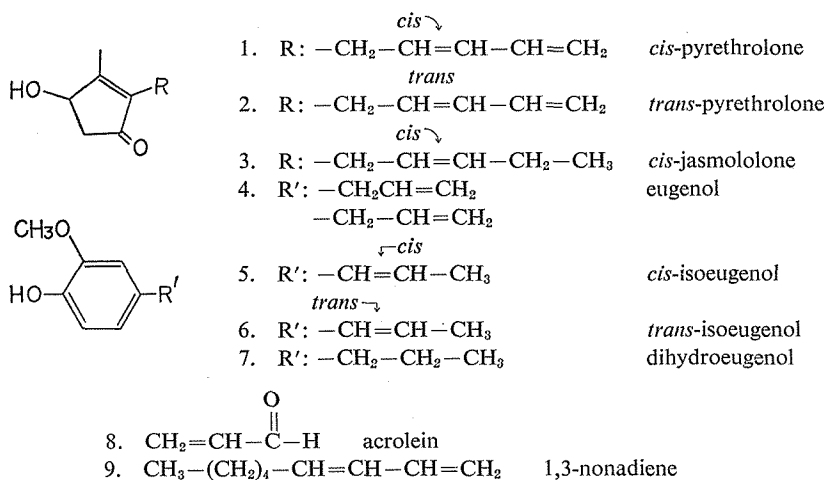
Table 2 Colorization of Compounds by Coloring Reagents.

| reagent compound | H ₃ PO ₄ —CH ₃ COOEt 4:1 by volume | H ₂ SO ₄ —CH ₃ COOH 20:1 by volume | HCl—CH ₃ COOH 1:1 by volume |
|---------------------|--|--|---|
| 1 | 520(5740) 550(6667) rose red | 514(1971) 550(2003) violet | 530(164) light pink |
| 2 | 550(2785) rose red | 518(660) 550(646) light pink | 540(175) dark violet |
| 3 | none colorless | none colorless | none colorless |
| 4 | 520(156)* deep red | 531(122)** deep red | none colorless |
| 5 | 395(117) 455(118) colorless | 520(144)** rose red | 540(7) light pink |
| 6 | 450(118) colorless | 525(199)** orange red | 532(30) red purple |
| 7 | none colorless | none colorless | none colorless |
| 8 | none colorless | none colorless | none colorless |
| 9 | none colorless | — | — |

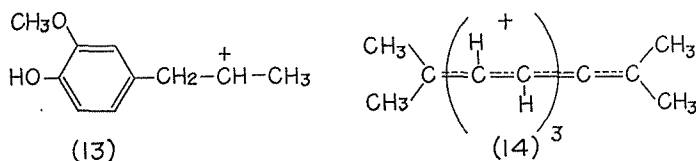
85%-Orthophosphoric acid, 98%-sulfuric acid and anhydrous acetic acid were used. * 85%-H₃PO₄ alone was used. ** H₂SO₄-CH₃COOH (1:1 by volume) was used. Wavelength (nm) and observed color were presented in boxes. Figures in parentheses represent ϵ_{max} .

The procedure was exemplified by reaction of eugenol with orthophosphoric acid reagent. To 5.0 g of eugenol, 100 ml of the reagent were added and the mixture was heated at 100°C for 10 min, then, a rose red color developed, and this reaction mixture was poured into 1000 ml of water and extracted with ether (100 ml×5); the color disappeared and the ether phase emitted a strong violet fluorescence. After removal of solvent, the distillation gave a colorless liquid. Separation and isolation by preparative g.l.c gave four fractions. The analysis of NMR and MASS spectra revealed these fractions to be the compounds (10), (11) and a mixture of (12) and *trans*-isoeugenol (6) respectively.

Color Reaction of Pyrethrins

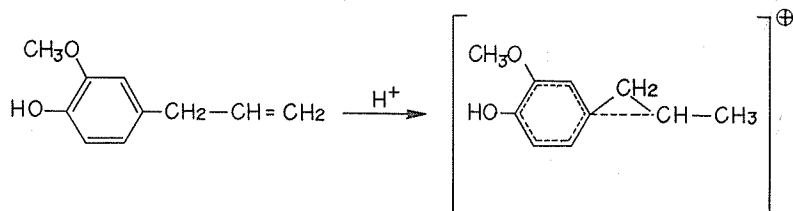


The formation of these compounds suggested that carbonium ion (13) was formed by protonation of allyl side chain in eugenol. To date, UV spectra of many polyenyl

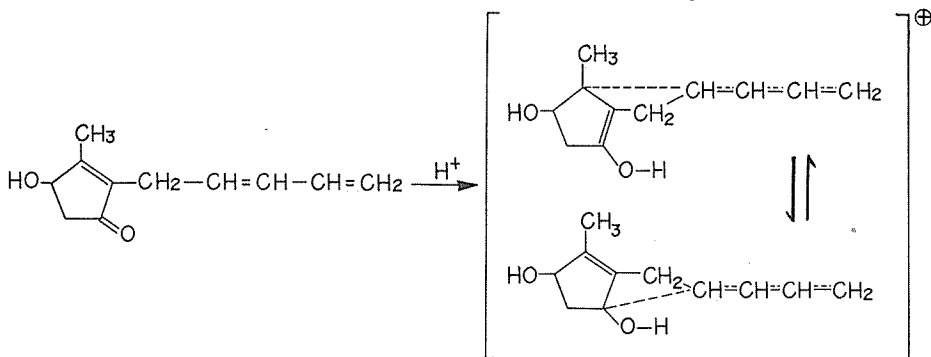


carbonium ions have been recorded by many workers.⁴⁻⁸⁾ For instance, pentaenyl carbonium ion (14) was found⁴⁾ to have λ_{max} at 550 nm, whereas the parent pentaene had λ_{max} in region of 250-375 nm. This red shift was explained on the basis of the free-electron theory⁹⁾ by the authors. Also, the fact that 1,1-bis-(4'-methoxyphenyl)-2,2-dimethyl-propylcarbonium ion has a rose red color⁵⁾ may be explained by delocalization. As another example, it was reported²⁾ that when some conjugated arylalkenes were treated with H₂SO₄-CH₃COOH, an anomalous absorption band appeared in a region of 600-700 nm. This absorption has been explained by the terms of pi-complex theory and, later, by the formation of conjugated aromatic radical cation from kinetic, spectrophotometric and magnetic evidences.^{2a)} However, it is not likely that carbonium ion (13) from eugenol has an absorption maximum in such a long wavelength region, since the orbital of this carbonium ion does not overlap through bond with any other conjugated systems. Accordingly, the origin of the color development can not be explained by such a delocalization, free electron theory, *etc.* Then, in the present case, a delocalization by 1,3-interaction¹⁰⁻¹²⁾ or homoconjugation was suggested for the

explanation of absorption in the long wavelength region. The interaction can be represented visually as follows:



Also, the coloring of pyrethrolone could not be explained from the same reason as in the case of eugenol since the alcohol has two chromophores, *viz.* acrolein and butadiene fragments, which were separated from each other by a methylene, and these two chromophores and its protonated species have no absorption in visible region. Accordingly, the color development of pyrethrolone in mineral acids was supposed also to originate in delocalization by 1,4-interaction¹²⁾ of these two chromophores by the acids as shown below.



Also, inductive and/or resonance effects of hydroxyl and methoxyl groups, and probably solvation might contribute to the red shift *viz.* stabilization of the excited states of these ions. It could be possible that the origin of color may be attributed to the formation of some charge transfer complex between alkenyl compounds (or its ions) and mineral acids (or its dissociated species), however, this problem is to be prosecuted further.

(II) Quantitative Determination of Natural Pyrethrins by the Use of the Color Reaction with the Orthophosphoric Acid Reagent

It was confirmed that pyrethrins I and II alone gave absorption maximum at 550 nm with the reagent (a mixture of 85%-orthophosphoric acid and ethyl acetate, 4:1 by volume) in the present research using pure materials obtained by synthesis. In addition, the analytical procedure was strictly specified. Since the total amount of insecticidal constituents in pyrethrum flowers should be estimated from the value found

for pyrethrins I and II in this method, the relative ratio of the six components in the standard solution and the test sample must be nearly the same. To examine the relative ratios, gaschromatography (5%-PEGs) was applied, and it was confirmed that the relative ratios were almost identical with respect to several samples of pyrethrum flowers of the same strain. Accordingly, using the analytical value obtained by acidimetry with an arbitrarily chosen sample, a calibration curve was drawn against the absorbance at 550 nm of the colored mixture with the reagent. One of the typical curve was shown in Figure 1. Then, the analytical procedure for dried pyrethrum flowers was specified strictly as follows.

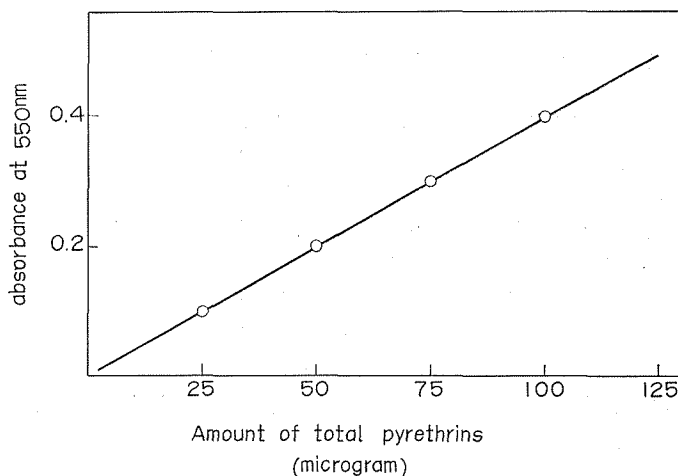


Fig. 1. Calibration Curve

(A) Preparation of sample solution

Extract ca. 3 g of dried and ground pyrethrum flowers with 160 ml of petroleum ether (b.p. 30–50°C) in a Soxhlet extractor (one cycle during 30 sec.) for 1 hr, concentrate the extract to about 4 ml at 50°C in water bath. Transfer this solution completely to a stoppered test tube (15 mm × 150 mm), and add 4 ml of nitromethane, shake for about one min. After standing still for several min, transfer nitromethane phase to a 250 ml measuring flask with a glass syringe. To the residual petroleum ether phase, add additional 4 ml of nitromethane, shake, and transfer the nitromethane phase to the measuring flask. Dilute the total nitromethane solution (8 ml) with acetone up to 250 ml. One ml of this solution (solution A) contains 30–90 micrograms of pyrethrins. Submit one ml of the solution A to the color reaction procedure which is carried out according to the same procedure as described below (B).

(B) Calibration curve

Pipette an aliquot of the standard* solution into an stoppered test tube (20 × 150 mm) and remove the solvent under reduced pressure at room temperature. Add 5 ml

* In the present research, an extract from pyrethrum flowers SHIRAYUKI of second grade (whose pyrethrins content was 1.40% by acidimetry) was used as a standard solution for calibration.

of the reagent to the solvent free sample and shake for one min. Immediately, heat the test solution at 100°C in a boiling water for 2.5 min and cool to room temperature with water. Measure absorbance as such at 550 nm using the color reagent as reference. Plot the absorbance ($\log I/I_0$) against the amount (microgram) of total pyrethrins. By these procedures, a fine linearity of calibration curve was obtained as shown in Fig. 1.

(C) Evaluation of total pyrethrins content in pyrethrum flowers sample

Using this curve, read the pyrethrins content (microgram, Y in the following expression) from the absorbance with a sample. Then, the total pyrethrins content (%) in dried pyrethrum flowers can be calculated by the expression:

$$\text{Total pyrethrins content (\%)} = \frac{Y}{40 \times X} (\%)$$

X: amount (g) of dried pyrethrum flowers taken (ca. 3 g)

By the procedure mentioned above, the pyrethrins content of three samples were determined in comparison with acidmetry. In Table 3 are listed the analytical results obtained. The values given in the column designated as method A were by the official acidmetry, while under B were summarized the values by the phosphoric acid-coloring method. Samples I, II and III were arbitrarily picked up from SHIRAYUKI crops harvested in June, 1971. As can be seen from the data, both methods A and B gave approximately the same values with all samples. The important feature of this colorimetric method is that it enables one to detect and determine pyrethrins on a microgram level within 3 hours.

Table 3 Analytical Data

| | I | II | III |
|---|------|------|------|
| A | 1.40 | 1.31 | 1.20 |
| B | 1.40 | 1.29 | 1.12 |

EXPERIMENTAL

Dried pyrethrum flowers were supplied by Dainippon Jotyūgiku Co., Ltd. eugenol and isoeugenol were by Ogawa Kōryō Co., Ltd. respectively, to whom the authors' thanks are due. UV spectra were taken on a Hitachi EPS-3 spectrophotometer, NMR spectra were on a Varian Model A-60 spectrometer with tetramethylsilane as the internal reference standard and chemical shifts were measured in ppm. IR spectra were on a Hitachi EPI-2 spectrophotometer. Gaschromatography were taken on a Hitachi K-53 with FID detector and a Varian Aerograph A-700 with TCD detector.

Jasmololone, jasmolins I and II, *cis*-pyrethrolone, *cis*-pyrethrins I and II were synthesized by the Crombie's methods,^{13,14)} in which *cis*-pentenyl side chain of jasmololone and *cis*-pentadienyl side chain of *cis*-pyrethrolone were introduced by the salt-free *cis*-selective Wittig reaction with propionaldehyde and acrolein respectively. *trans*-Pyrethrolone was prepared according to the method of Crombie *et al.*,¹⁶⁾

in which the *trans* structure of side chain in pyrethrolone was stereospecifically formed by the Knoevenagel reaction.^{17,18)} The structure and integrity of these materials were identified authentically by the coincidence of IR and NMR spectra with those in the Crombie's literature.¹⁵⁾

Spectral data

jasmololone: IR ν_{\max}^{liq} cm^{-1} : 3400, 1700, 1645, 1050 and 1010. NMR (CDCl_3) δ : 1.0 (3H, t, $-\text{CH}_3$ in pentenyl side chain), 1.8–2.4 (2H, m, methylene protons on 4' carbon in pentenyl side chain), 2.1 (3H, s, $-\text{CH}_3$ on cyclopentenolone ring), 2.0–2.8 (2H, m, ring methylene), 2.75 (1H, s, $-\text{OH}$), 2.9 (2H, d, $J=7$ Hz, methylene protons on 1' carbon in pentenyl side chain), 4.6–4.8 (1H, m, ring methine proton).

jasmolin I: IR ν_{\max}^{liq} cm^{-1} : 2950, 2850, 1790, 1730, 1715, 1660, 1380, 1193, 1155, 995 and 860. NMR (CDCl_3) δ : 1.75 (6H, s, two terminal methyl groups in chrysanthemic acid side chain), 1.15 (3H, s, methyl group *trans* to carboxyl in cyclopropane ring), 1.3 (3H, s, methyl group *cis* to carboxyl group on cyclopropane ring), 2.0 (3H, s, $-\text{CH}_3$ on cyclopentenolone ring), 1.0 (3H, s, $-\text{CH}_3$ in pentenyl side chain).

jasmolin II: IR ν_{\max}^{liq} cm^{-1} : 2930, 2850, 1780, 1720, 1690, 1650, 1380, 1285, 1195, 1155, 1115 and 855. NMR (CDCl_3) δ : 3.7 (3H, s, $-\text{COOCH}_3$ in pyrethric acid moiety), 1.25 (3H, s, $-\text{CH}_3$ *trans* to $-\text{COOR}$ on cyclopropane ring), 1.3 (3H, s, $-\text{CH}_3$ *cis* to $-\text{COOR}$ on cyclopropane ring), 2.0 (3H, s, $-\text{CH}_3$ on cyclopentenolone ring), 1.0 (3H, $-\text{CH}_3$ in pentenyl side chain).

pyrethrolone: IR ν_{\max}^{liq} cm^{-1} : 3450, 1820, 1685, 1643, 1090, 1050, 1005 and 908. NMR (CDCl_3) δ : 5.0–7.0 (5H, m, $-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2$), 2.1 (3H, s, $-\text{CH}_3$ on cyclopentenolone ring), 3.4 (1H, s, $-\text{OH}$), 4.6–4.8 (1H, m, ring methine proton), 3.1 (2H, d, $J=6$ Hz, $-\text{CH}_2-$ in pentadienyl side chain), 2.0–2.8 (2H, dd, ring methylene protons).

pyrethrin I: IR ν_{\max}^{liq} cm^{-1} : 1725, 1712, 1660, 1595, 995 and 905. NMR (CDCl_3) δ : 1.15 (3H, s, $-\text{CH}_3$ *cis* to $-\text{COOR}$ on cyclopropane ring), 1.3 (3H, s, $-\text{CH}_3$ *cis* to $-\text{COOR}$ on cyclopropane ring), 1.7 (6H, s, two terminal methyl groups in chrysanthemic acid side chain), 2.0 (3H, s, $-\text{CH}_3$ on cyclopentenolone ring), 3.1 (2H, d, $J=6$ Hz, $-\text{CH}_2-$ in pentadienyl side chain), 5.0–7.0 (5H, m, $-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2$).

pyrethrin II: IR ν_{\max}^{liq} cm^{-1} : 2900, 1725, 1713, 1965, 1645, 1430, 1380, 1190, 1155, 1115 and 830. NMR (CDCl_3) δ : 1.15 (3H, s, $-\text{CH}_3$ *trans* to $-\text{COOR}$ on cyclopropane ring), 1.3 (3H, s, $-\text{CH}_3$ *cis* to $-\text{COOR}$ on cyclopropane ring), 3.7 (3H, s, $-\text{COOCH}_3$ in pyrethric acid moiety), 2.05 (3H, s, $-\text{CH}_3$ on cyclopentenolone ring), 3.1 (2H, d, $J=5$ Hz, $-\text{CH}_2-$ in pentadienyl side chain), 5.0–7.0 (5H, m, $-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2$).

trans-pyrethrolone: IR ν_{\max}^{liq} cm^{-1} : 3400, 1800, 1700, 1645, 1050, 1005 and 960. NMR (CDCl_3) δ : 2.15 (3H, s, $-\text{CH}_3$ on cyclopentenolone ring), 4.85 (1H, m, methine proton in cyclopentenolone ring), 2.7 (1H, s, $-\text{OH}$), 3.1 (2H, d, $J=6$ Hz, $-\text{CH}_2-$ in side chain), 5.2–6.9 (5H, m, $-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2$).

trans-isoeugenol: This was isolated pure by preparative g.l.c (15%-Reoplex-400) from a mixture of *cis*- and *trans*-isoeugenol. IR ν_{\max}^{liq} cm^{-1} : 3550, 1720, 1600, 1030, 960, 855 and 800. NMR (CDCl_3) δ : 1.83 (3H, d, $J=5$ Hz, $>\text{C}-\text{CH}_3$), 3.87 (3H, s, $-\text{OCH}_3$), 5.55 (1H, s, $-\text{OH}$), 6.0–6.6 (2H, m, $-\text{CH}=\text{CH}-$), 6.83 (3H, s, phenyl protons).

cis-isoeugenol: This was also isolated from the *cis*- and *trans*-mixture on the same

column. IR ν_{\max}^{liq} cm^{-1} : 3500, 1720, 1595, 1605, 1510, 1420, 1265, 1030, 860 and 820. NMR (CDCl_3) δ : 1.9 (3H, dd, $J=1.5$ and 7 Hz, $>\text{C}-\text{CH}_3$), 3.9 (3H, s, $-\text{OCH}_3$), 5.6 (1H, s, $-\text{OH}$), 5.5–6.0 (1H, m, vinyl proton), 6.35 (1H, dd, $J=1.5$ and 12 Hz, vinyl proton), 6.7–6.9 (3H, m, phenyl protons).

dihydroeugenol: Eugenol (5g, 0.03 mol) was hydrogenated using palladium (1.0 g) on barium sulfate (20 g) in usual manner and distillation gave pure dihydroeugenol. b.p. 87–88°C/1.5 mm. IR ν_{\max}^{liq} cm^{-1} : 3515, 1605, 1510, 1460, 1430, 1270, 1230, 1205, 1035, 930, 815, 795 and 777. NMR (CDCl_3) δ : 0.9 (3H, t, $J=6$ Hz, $-\text{CH}_3$ in *n*-propyl group), 1.3–1.9 (2H, m, $-\text{CH}_2-$), 2.53 (2H, t, $J=7$ Hz, $\text{Ph}-\text{CH}_2-$), 3.85 (3H, s, $-\text{OCH}_3$), 5.48 (1H, s, $-\text{OH}$), 6.5–7.0 (3H, m, phenyl protons).

1,3-nonadiene: The Wittig reaction of the phosphorane from *n*-hexyltriphenylphosphonium bromide (21.4 g, 0.05 mol) and acrolein (2.8 g, 0.05 mol) gave 1,3-nonadiene¹⁹⁾ (4.0 g). b.p. 70–71°C/44 mm. IR ν_{\max}^{liq} cm^{-1} : 3100, 3010, 2950, 2870, 1810, 1640, 1590, 1460, 1430, 1379, 996, 900, 785 and 730. NMR (CDCl_3) δ : 0.9 (3H, t, $J=7$ Hz, terminal $-\text{CH}_3$), 1.1–1.7 (6H, m, $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$ methyl), 1.9–2.5 (2H, m, $-\text{CH}_2-\text{C}=\text{C}-$), 4.8–9.5 (5H, m, $-\text{CH}=\text{CH}-\text{CH}=\text{CH}_2$).

Isolation and structural elucidation of the reaction products from eugenol and color reagent

Gaschromatogram of the distillate (b.p. 95–132°C/2 mm) showed four peaks at (A) 4 min (B) 6.5 min (C) 16.5 min and (D) 20.5 min through 15%-Reoplex-400. Of these four, it was found by the analysis of IR, NMR and MASS spectra that fraction (A) was unreacted eugenol, (B) was a mixture of *trans*-isoeugenol and the compound (12), (C), the compound (11) and (D), the compound (10).

Spectral data of compounds (10)–(12)

(10): IR ν_{\max}^{liq} cm^{-1} : 3495, 1600, 1513, 1450, 1430, 1370, 1270, 1235, 1205, 1155, 1120, 1035, 940 and 810. NMR (CDCl_3) δ : 1.23 (3H, d, $J=6$ Hz, terminal methyl), 1.7 (1H, s, $>\text{C}-\text{OH}$), 2.65 and 2.67 (2H, d and d, $J=8$ and 4 Hz, $\text{Ph}-\text{CH}_2-$), 3.87 (3H, s, $-\text{OCH}_3$), 3.6–4.2 (1H, m, $>\text{CH}-\text{O}-$), 5.65 (1H, s, $\text{Ph}-\text{OH}$), 6.5–7.0 (3H, m, phenyl protons). MASS (m/e): 39, 45, 51, 65, 77, 94, 106, 137, 182 (molecular ion). (11): IR ν_{\max}^{liq} cm^{-1} : 3495, 1730, 1512, 1430, 1370, 1155, 1125, 1035, 957, 815 and 795. NMR (CDCl_3) δ : 1.23 (3H, d, $J=6$ Hz, terminal methyl), 2.0 (3H, s, $-\text{CO}-\text{CH}_3$), 2.72 and 2.80 (2H, d and d, $J=6$ and 6 Hz, $\text{Ph}-\text{CH}_2-$), 3.87 (3H, s, $-\text{OCH}_3$), 5.1 (1H, dd, $J=6$ and 6 Hz, $>\text{CH}-\text{O}-$), 5.51 (1H, s, $\text{Ph}-\text{OH}$) and 6.6–7.0 (3H, m, phenyl protons). MASS (m/e): 77, 94, 105, 122, 137, 149, 164, 224 (molecular ion). (12): IR spectrum of this fraction closely resemble that of *trans*-isoeugenol. NMR (CDCl_3) δ : 1.15 (3H, d, $J=6$ Hz, $>\text{C}-\text{CH}_3$), 1.20 (3H, t, $J=7$ Hz, $-\text{CH}_3$ in $-\text{O}-\text{CH}_2-\text{CH}_3$), 2.65 and 2.80 (2H, d and d, $J=7$ and 6 Hz, $\text{Ph}-\text{CH}_2-$), 3.3–3.8 (1H, dd, $J=7$ Hz, $>\text{CH}-\text{O}-$), 5.7 (1H, s, $\text{Ph}-\text{OH}$) and 6.8–7.1 (3H, m, phenyl protons). Other signals in this chart coincided with those of *trans*-isoeugenol. In the MASS spectrum of the fraction (B), a peak appeared at 210 m/e which was considered to be molecular ion peak of (12).

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